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(FY 91 AASERT) MOLECULAR PROBES AND BIOLUMINESCENT REPORTERS IN ECOLOGICAL OPTIMIZATION OF BIODEGRADATION

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**13. ABSTRACT (Maximum 200 words)**

DNA probe and bioluminescent sensor technology is being used to assess the bioabailability of sorbed or immiscible-phase toluene and polycyclic aromatic hydrocarbons (PAH) in particulate media. Construction of improved bioluminescent reporter strains for PAH and toluene (also trichloroethylene) degradation is ongoing. For PAH degradation, the approach involves incorporation of a transposon containing the lower naphthalene pathway promoter fused to the lux genes (nah-lux) into the bacterial chromosome. One of the two transposons (Tn5-based transposon) appears to be successful in forming the fusion product and incorporating into the Psuedomonas genomes. Work is ongoing. For toluene biodegradation, the approach involves a bacterial strain containing a plasmid-encoded tod-lux gene fusion. The strain produces light when the inducer, toluene, signals an increased production of the catabolizing enzyme, toluene diooxygenase. Finally, a related project involves the development of a combined method to extract and analyze both DNA and lipids from the same environmental sample in order to maximize the informational content of a single sample with respect to biomass content, community structure and the physiological status of microorganisms.

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Contract Number F49620-92-J-0333
Final Technical Report (June 1, 1994 - May 31, 1995)
(FY91 AASERT) Molecular Probes and Bioluminescent Reporters in Ecological Optimization of Biodegradation

G.S. Sayler
Center for Environmental Biotechnology
University of Tennessee, Knoxville

The focus of research supported by this grant is to apply molecular biological approaches including DNA probe and bioluminescent sensor technology to problems in organic contaminant biodegradation. The specific research issue addressed in this project is the role that surfactants play in enhancing the bioavailability of sorbed or immiscible-phase polycyclic aromatic hydrocarbons (PAHs) in particulate media. Increased bioavailability is assessed in terms of increased PAH-degrader population densities (determined from DNA hybridization analysis) and their activities including the rate and extent of biodegradation and gene expression as measured by bioluminescence response and mRNA levels.

Bacterial strains containing specific degradative genes and bioluminescent reporter systems are being used to monitor the effectiveness of surfactants for enhancing contaminant biodegradation. Construction of an improved bioluminescent reporter strain for PAH degradation is ongoing. This approach involves incorporation of a transposon containing the lower naphthalene pathway promoter fused to the *lux* genes (*nah-lux*) into the bacterial chromosome. To date, a *nah-lux* fusion was constructed on a *Tn1721*-based transposon in *E. coli*. Attempts were made to incorporate the transposon into the chromosome of a *Pseudomonas* strain by either electroporation or conjugation (after transposition into a matable vector). Since several attempts have been unsuccessful, the *nah-lux* fusion is also being constructed on a *Tn5*-based transposon which has been shown to transpose and stably insert into *Pseudomonas* genomes. This work is in the last stage of construction and the *nah-lux* reporter strain should be in hand soon. The strain lacking the *nah*-type plasmid will be used as a control strain to account for light production not correlated with gene expression. By monitoring the light response of the reporter and control strains, definitive results concerning increased PAH bioavailability (or potential toxic effects) due to the surfactant treatments will be obtained. While this work will continue with other support, published information will acknowledge the major support provided the Air Force AASERT.

Currently, a strain containing a plasmid-encoded *tod-lux* gene fusion has been used. The use of this strain has allowed for the optimization of the bioluminescent assays for assessing bioavailability. This strain produces light in the presence of the inducer, toluene, while the toluene dioxygenase can catabolize TCE and toluene. Therefore, an increased light response represents an increased production of the enzyme which catabolizes TCE. This strain will respond to concentrations as low as 0.1 ppm toluene. Analysis of mRNA showed that *lux* gene expression does not increase above 1 ppm toluene while *tod* gene expression continues to increase to 10 ppm toluene. It appears that the regulatory protein may be titrated out by the multiple copies of the *tod* promoter fused to the *lux* gene cassette. Therefore, a chromosomally-based *tod-lux* fusion similar to that described for *nah-lux* is also being constructed and is nearly completed.

A related project has involved development of a combined method to extract and analyze both DNA and lipids from the same environmental sample. Lipid analysis provides complementary information to DNA analysis with respect to biomass content and community structure and also additional information with respect to physiological status of microorganisms. DNA which was extracted by this method was quantified using DNA:DNA hybridization analysis with *nahA* (naphthalene dioxygenase) or *todC1* (toluene dioxygenase) gene probes. Estimates of population densities based on either lipid or DNA analysis showed good agreement with the amount of cells added to the soil. This work resulted in a poster presentation at the Third International Symposium on the Interface between Analytical Chemistry and Microbiology (Knoxville, TN, March 12-16, 1995). The manuscript describing the approach has been accepted for publication in the Journal for Microbiological Methods.

Academic Progress

The student supported on Contract No. F49620-92-J-0333, Staci R. Kehrmeyer has made satisfactory academic progress towards her Ph.D. requirements. The following courses were taken with the appropriate letter grades indicated below:

Academic Years 1992-1995
Biochemistry 511-Advanced Concepts in Protein Structure, Protein Function and Intermediary Metabolism (A)
Biochemistry 512-Advanced Molecular Biology (A)
Microbiology 470-Microbial Ecology (A)
Microbiology 670-Advanced Topics in Environmental Microbiology (A)
Mathematics 405-Models in Biology (A)
Chemistry 431-Radioactive Tracer Techniques (A)
Geology 485-Principles in Geohydrology (A)
Microbiology 670-Advanced Topics in Environmental Microbiology (B+)

The student completed all necessary courses toward the Ph.D. degree and passed her qualifying examination.